

# Studies on blood coagulation factor V

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# Studies on blood coagulation factor V

## II — Preparation and properties of an artificial factor V reagent by adsorption with Ba-stearate<sup>(1)</sup>

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To estimate factor V, three types of reagent can be used: old oxalated plasma (Quick 1943, plasma incubated with Russell's viper venom (R.V.V.) (Borchgrevink 1960) or congenitally deficient plasma. Old plasma exhibits variable properties, which made it an inconvenient reagent in our hands. R.V.V.-treated plasma is satisfactory for routine purposes, but for a study of the kinetics of factor V action it is less satisfactory because a procoagulant alien to normal plasma is added. Congenitally deficient plasma is the most desirable reagent, but it is extremely scarce. We therefore decided to exploit the property of factor V to be adsorbed by Ba-stearate (Vroman 1958) for the preparation of factor V reagent. The differences in the adsorption-characteristics of factor V and the other factors, however, are not sufficiently large, even under the most favourable circumstances for mere adsorption to yield a useful factor V reagent (Kahn 1969), because complete removal of factor V is only brought about by concentrations of Ba-Stearate that induce considerable losses of the coagulation factors XII, X, VII and I.

### MATERIALS AND METHODS

Preparation of starting materials and determination of coagulation factors was carried out as indicated in the next section. Fibrinogen was estimated according to Clauss (Clauss 1957). Thromboplastin time is measured in a mixture of 0.1 ml plasma sample, 0.1 ml human brain thromboplastin, and 0.1 ml 33 mM  $\text{CaCl}_2$ , at 37°C. The

factor V reagent according to Borchgrevink is prepared as indicated in ref. 1. It is used in a reaction mixture consisting of 0.1 ml sample (diluted so as to contain between 1 and 10 % factor V), 0.1 ml reagent, 0.1 ml human brain thromboplastin, and 0.1 ml  $\text{CaCl}_2$  25 mM.

This reaction mixture was also used when the factor V reagent described in this article was employed. All clotting time estimations were done in fourfold. Clotting factor concentrations were read from a reference curve obtained by determining the coagulation times of a dilution series of normal plasma (concentrations: 10 %, 5 %, 3.3 %, 2.5 %, 2 %, 1.7 %, 1.4 %, 1.2 %, 1 %). The human brain thromboplastin was prepared according to Owren and Aas (1951). The normal plasma pool was obtained from 30 healthy normals, mean age 32 yrs [18-43] and stored at -70°C.

The congenitally factor V-deficient plasma was a kind gift of the plasma of Owren's original patient by Prof. Dr H. Stormorken.

### PREPARATION OF THE REAGENT

Human citrated plasma is mixed with 50 mg/ml Ba-Stearate (K & K Laboratories, Plainview, New York) in a 50 ml glass Potter-Elvehjem homogenizer with an electrically driven teflon pestle. The mixture is left at 37°C for one to two hours. In one session 500 to 1000 ml plasma can be processed. The mixture is centrifuged at 25,000 g for 20 min. at 4°C. A floating sediment is removed by suction. The supernatant is decanted. It is not entirely clear, but becomes clear after filtration (filter paper no 606 $\frac{1}{2}$ , Macherey Nagel & Co., Dürer, Germany). The plasma has a pH of  $8.1 \pm 0.1$ ; it is brought to pH 5.4 with 1N HCl. The acidified plasma is incubated at 37°C.

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(1) Premier texte à paraître in *Thromb. Diath. Haemorrh.*

**Table I**  
CONCENTRATION OF CLOTTING FACTORS DURING PREPARATION OF THE FACTOR V REAGENT

Stage of preparation	pH	Concentration of coagul. factor				
		I	II	V	VII/X	X
Starting plasma .....	7.8	100	100	100	100	100
After adsorption with Ba-Stearate .....	8.1	92	100	15	70	60
After acidification :						
0 min. incubation .....	5.4	92	100	5.0	70	60
10 min. incubation .....	5.4	92	100	4.0	70	57
30 min. incubation .....	5.4	85	100	1.0	70	57
60 min. incubation .....	5.4	80	100	0.3	74	54
70 min. incubation .....	5.4	80	92	0.1	70	51
After 6 months storage at —20 °C .....	7.4	80	91	0.2	73	50

The values represent percentages of the starting material. All estimations were done after adjustment of the pH to 7.4.

Corrections were carried out for the dilution brought about by adjustment of the pH.

Every 10 min. a sample is drawn and adjusted to pH 7.4 with N.NaOH, after which a thromboplastin time determination is carried out. After about 60 min. this time has become longer than 70 seconds. The factor V content estimated according to Borchgrevink will then appear to be less than one percent. When a sample is drawn that shows a thromboplastin time of more than 70 seconds, the whole batch is brought to pH 7.4. The reagent is stored in 1 or 2 ml portions in plastic tubes at —20° centigrade (the reagent is stable for at least 6 months).

Table I gives the determinations of individual coagulation factors at the various stages of the preparation.

## PROPERTIES OF THE REAGENT

The mean values of the concentrations of the coagulation factors in 6 different batches of the reagent were : Factor I : 2.75 mg/ml ; factor II : 62 % ; factor V : 0.25 % ; factor VII : 59 % ; factor VIII : 30 % ; factor IX : 36 % ; factor X : 60 % ; factor XI : 20 % ; factor XII : 95 % ; anti-thrombin III : 56 %. Table II shows that the defect in the reagent is restored by normal plasma and by various adsorbed plasmas, but not by old oxalated plasma or congenitally factor V deficient plasma. The table also shows that the reagent reacts qualitatively like Borchgrevink's reagent in this respect. This lends sufficient support to the opinion that it is factor V that is specifically lacking in the reagent.

To be useful, a reagent must not be influenced by changes in the concentrations of coagulation factors for which it is not supposed to be specific. Fig. 1 shows that when tested in individual plasmas there is no correlation bet-

**Table II**  
CORRECTION OF COAGULATION TIME OF THE REAGENT BY VARIOUS PLASMAS

Plasma added	Coagulation time obtained with	
	Ba-Stearate adsorbed plasma	Borchgrevink's reagent
Normal citrated plasma	25	17
BaSO <sub>4</sub> .....	24	17,5
Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub> adsorbed plasma .....	24,5	18
Al(OH) <sub>3</sub> adsorbed plasma	29	18
Old oxalated plasma ....	48	39
Factor V deficient plasma	53	42
Michaelis buffer (pH 7.4)	70	62

Coagulation times in seconds. Means of 6 estimations with two different batches of reagent.

Reaction mixture : 0.1 reagent, 0.1 ml plasma or buffer, 0.1 ml human brain thromboplastin, 0.1 ml CaCl 25 mM.

ween factor V concentration as measured with our reagent and factor I concentration. Fig. 2 shows that the apparent factor V concentration is not influenced by extreme variations in the concentration of factors II, VII, IX and X. was brought about by mixing normal plasma with an equal volume of varying mixtures of Michaelis buffer and a preparation (P.P.S.B.) containing 800 % factor VII/X, 630 % factor II, and 680 % factor IX. The final concentration in the sample of the factors VII and X (estimated in combination) is rendered on the X axis. Variations



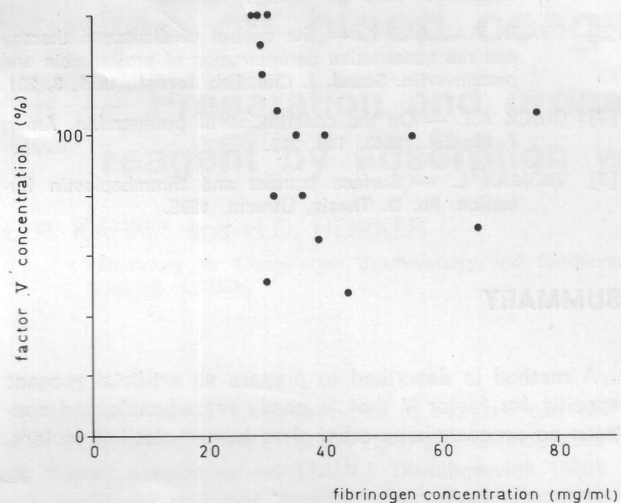


Fig. 1. — Correlation between fibrinogen content and factor V concentration as estimated with Ba-Stearate adsorbed plasma.

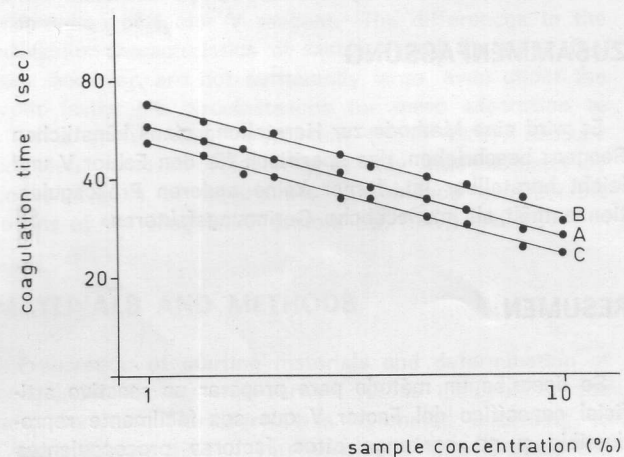


Fig. 3. — The reference curve of the factor V estimation.

- A. Normal pooled plasma.
- B. Plasma with 50 % factor V.
- C. Plasma with 140 % factor V.

On the X axis the concentration of the sample is plotted as a percentage of the starting material, therefore only in line A the percentages indicated coincide with real percentages of factor V. Each point is the mean of 10 determinations.

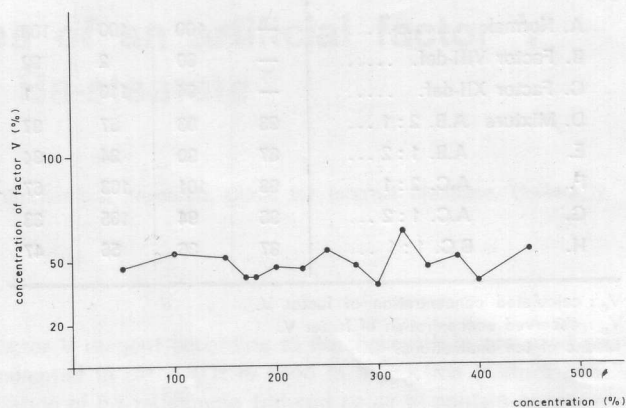


Fig. 2. — Correlation between the concentration of factors II, VII, IX and X (rendered on the abscissa) and the factor V concentration as measured with Ba-Stearate adsorbed plasma (determinations in triplo).

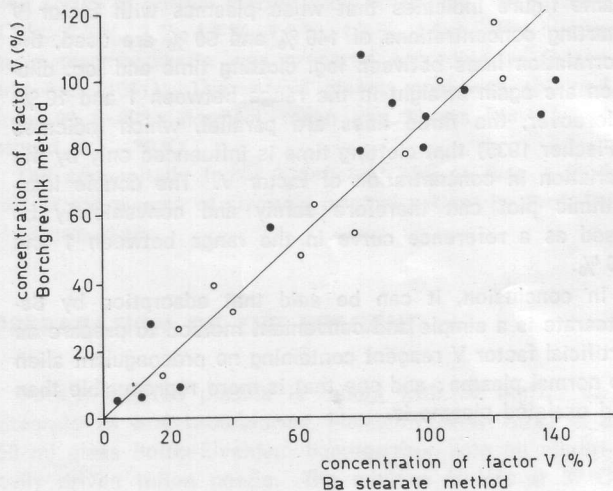


Fig. 4. — Correlation between two methods of factor V estimation. Each point refers to a plasma from a different patient. Each estimation was carried out in quadruplo.

- dilutions of a given plasma
- individual plasmas

Table III

INFLUENCE OF THE AMOUNT OF FACTORS VIII AND XII ON THE ESTIMATION OF FACTOR V

Plasma	Concentration of factor			
	V <sub>c</sub>	V <sub>o</sub>	VIII	XII
A. Normal .....	—	100	100	100
B. Factor VIII-def. ....	—	80	2	92
C. Factor XII-def. ....	—	94	110	1
D. Mixture A.B. 2:1 ...	93	93	67	97
E. A.B. 1:2 ...	87	90	34	94
F. A.C. 2:1 ...	98	101	103	67
G. A.C. 1:2 ...	96	94	105	33
H. B.C. 1:1 ...	87	86	56	47

V<sub>c</sub>: calculated concentration of factor V.

V<sub>o</sub>: observed concentration of factor V.

Means of ten estimations.

between 1 and 100 % of factor VIII and factor XII in the sample also failed to influence the factor V content estimated by this method (table III).

Fig. 3 shows that when normal plasma is tested in different dilutions the plot of the logarithm of the dilution against the logarithm of the clotting time is a straight line in the range of concentrations between 1 and 10 %. The same figure indicates that when plasmas with factor V starting concentrations of 140 % and 50 % are used, the correlation lines between log. clotting time and log. dilution are again straight in the range between 1 and 10 %. Moreover, the three lines are parallel, which indicates (Fischer 1935) that clotting time is influenced only by the variation in concentration of factor V. The double logarithmic plot can therefore safely and conveniently be used as a reference curve in the range between 1 and 10 %.

In conclusion, it can be said that adsorption by Ba-Stearate is a simple and convenient method to prepare an artificial factor V reagent containing no procoagulant alien to normal plasma; and one that is more reproducible than old oxalated plasma is.

## REFERENCES

- [1] BORCHGREVINK C.F., POOL J.G., STORMORKEN H. — A new assay for factor V (proaccelerin - accellerin) using Russell's viper venom. *J. Lab. Clin. Med.*, 1960, **55**, 625.
- [2] CLAUSS A. — Gerinnungsphysiologische Schnellmethode zur Bestimmung des Fibrinogens. *Acta Haemat.* (Basel), 1957, **17**, 237.

- [3] FISCHER R.A. — The design of experiments. Oliver and Boyd, 1935, 1<sup>st</sup> ed..
- [4] KAHN M.J.P., HEMKER H.C. — Studies on the blood coagulation factor V. I: The interaction of salts of fatty acids and coagulation factors. *Thromb. Diath. Haemorrh.*, 1970 (in press).
- [5] OWREN P.A., AAS K. — The control of dicumarol therapy and the quantitative determination of prothrombin and proconvertin. *Scand. J. Clin. Lab. Invest.*, 1951, **3**, 201.
- [6] QUICK A.J. — On the constitution of prothrombin. *Amer. J. Physiol.*, 1943, **140**, 212.
- [7] VROMAN L. — Surface contact and thromboplastin formation. *Ph. D. Thesis*, Utrecht, 1958.

## SUMMARY

A method is described to prepare an artificial reagent specific for factor V that is easily reproducible and contains no procoagulants other than human clotting factors.

## RESUME

Une méthode est décrite pour préparer un réactif artificiel spécifique du Facteur V qui soit facilement reproductible et ne contienne aucun autre procoagulant que les facteurs de coagulation humains.

## ZUSAMMENFASSUNG

Es wird eine Methode zur Herstellung eines künstlichen Reagenz beschrieben, das spezifisch für den Faktor V und leicht herstellbar ist, ferner keine anderen Prokoagulantien enthält als menschliche Gerinnungsfaktoren.

## RESUMEN

Se describe un método para preparar un reactivo artificial específico del Factor V que sea fácilmente reproductible y no contenga otros factores procoagulantes distintos de los de coagulación humanos.

"Исследование Фактора V коагуляции крови"

**Резюме** Описывается метод приготовления искусственного реагента специфического для фактора V, т.е. легко воспроизводимого и не содержащего никаких прокоагулянтов за исключением свертывающих факторов.